NEMATODE PARASITES OF THE KANGAROO ISLAND WALLABY, *MACROPUS EUGENII* (DESMAREST) I. SEASONAL AND GEOGRAPHICAL DISTRIBUTION

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Summary

SMALES, L. R. & MAWSON, P. M. (1978) Nematode parasites of the Kangaroo Island Wallaby, *Macropus eugenii* (Desmarest). I. Seasonal and geographical distribution. *Trans. R. Soc. S. Aust.* **102**(1), 9-15, 28 February, 1978.

The helminths of *Macropus eugenii* in Kangaroo I. have been identified at least to genus. Comparison of species collected in one four-day period from four areas, differing geographically and ecologically, show differences in their occurrence.

The incidence of strongyle nematodes in the stomach and of their eggs in the faeces, checked at 2-monthly intervals over two years, shows seasonal variation both of total numbers and of the constituent species, and this is linked with climatic conditions suitable for reinfestation with the worms.

Introduction

A systematic survey of the helminths of the Kangaroo Island Wallaby *Macropus eugenii* (Desmarest) was undertaken preliminary to work (Smales 1976[1]) on the life history of the stomach strongyle, *Labiostrongylus eugenii* (Johnston & Mawson, 1940a).

The wallaby is one of the smallest macropods. Its distribution is now limited to small populations on the mainland of south-western Australia and to some off-shore islands, the largest of which is Kangaroo Island. The hosts for this study were taken from Kangaroo Island, where they are still present in large numbers.

The climate of Kangaroo Island is the Mediterranean type, with most rain falling in winter. The mean monthly maximum and minimum temperatures and monthly rainfall recorded at Kingscote (April 1972–August 1972) or at Parndana (September 1972–later) were obtained from the Adelaide Bureau of Meteorology (Fig. 1). There appears to have been little, if any, variation, in the temperature for each month over these four years, though there is some variation in rainfall—the September fall being low in 1972, the July fall high in 1974. The general pattern of winter rain and summer dryness was maintained throughout the four year period.

The vegetation of Kangaroo Island varies from wet sclerophyll forest to open grassland with scrub, the preferred habitat of the wallaby being dry sclerophyll forest with dense undershrubs. This species appears to be specialised for life under conditions of much less rainfall than would support the rainforest or wet sclerophyll forest where other wallaby species are found (Ride 1970).

The wallabies were collected from one or more of four farm properties, each in a different part of the island, and with different plant associations. At Brookland Park, on the central plateau, with a rainfall of 831 mm a year, a mallee form of *Eucalyptus remota* is dominant. Around Pioneer Bend, also on the central plateau, with 559-584 mm of rain a year, *E. obliqua* and *E. cosmophylla* are the dominants. At Cape Cassini on the northern marginal slopes of the plateau, 559-584 mm of rain, *E. cneorifolia* is the dominant, associated with *Melaleuca uncinata*. An association of *E. diversifolia* and *E. rugosa*, with *E. diversifolia* in different forms of mallee ranging from low

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[1] Smales, L. R. (1976) A study of the biology of a nematode *Labiostrongylus eugenii* (Johnston & Mawson) parasitic in the stomach of the tammar wallaby (*Macropus eugenii* (Desmarest)). Ph.D. Thesis, University of Adelaide (unpublished).

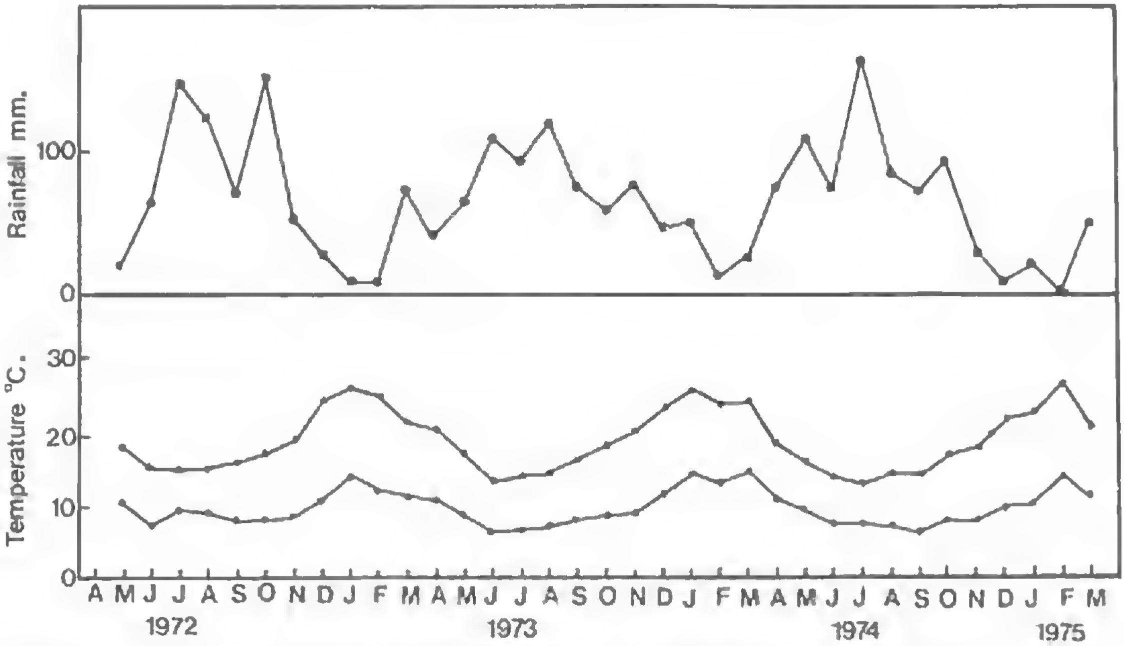

Fig. 1. Maximum and minimum average temperature and rainfall for 1972-1975, Kangaroo Island. From September 1972 taken at Parndana, previously at Kingscote.

tangled shrubs to trees 7.6 m high, is found at Nepean Bay in the Nepean Embayment lowland plain, where there is only 483-508 mm of rain. The species of undershrubs found with each plant association vary and are controlled for the most part by edaphic rather than climatic factors[2].

Stands of virgin bush have been left on each of the farms and these are often sufficiently large to provide shelter for the wallabies, which require the protection of dense undershrubs through the day, and come out into the farm paddocks at night to feed. Farming procedures were similar on all properties except that the clover fields at Brookland Park and Pioneer Bend were sprayed in August–October of 1973 and 1974 to control Black Spot disease, *Kabatiella* sp., with Thibenzole (Merck, Sharp & Dohme). Before this no spray had been used.

At autopsy of each wallaby the stomach wall was checked for lesions, and a low 10% (by volume) sample of the stomach contents taken. This sample as well as the intestinal tract, liver, and thorax, were examined for helminths, which were collected and fixed in hot 10% alcohol or 4% formalin, and later identified and counted.

Geographical variation

In view of the varying rainfall and vegetation in different parts of Kangaroo Island, we sampled and compared the helminth fauna of animals from different areas at more or less the same date.

Animals were collected from each of the four areas described above on four consecutive nights in April 1975. Autumn was chosen because the heaviest infestations had previously been observed during autumn and winter. Up to 10 wallabies were shot in each area. Each animal was weighed, sexed, and its age determined from the tooth eruption pattern.[1]

No helminths were found in the bile ducts or liver. The species identified were: from the thoracic cavity, the nematode *Dipetalonema* sp.; from the intestine, the nematodes *Globocephaloides trifidospicularis* Kung 1948 and *Austrostrongylus thylogale* Johnston & Mawson 1940, and the cestodes *Triplotaenia* sp., and *Progamotaenia* spp. A & B; from the stomach, the nematodes *Cloacina* spp., *Labiostrongylus eugenii* (Johnston & Mawson, 1940a), *L. longispicularis* Wood 1929, *Macropostrongylus pearsoni* (Johnston & Mawson, 1940b), *Oesophagonastes kartana* (Mawson,

[2] Bauer, F. H. (1959) The regional geography of Kangaroo Island South Australia. Ph.D. Thesis. Australian National University (unpublished).

TABLE 1
Helminths collected from *M. eugenii* on Kangaroo Island during April 1975

Number of wallabies	Nepean Bay 10		Cape Cassini 10		Pioneer Bend 8		Brookland Park 10	
	No. infected	Mean No. worms	No. infected	Mean No. worms	No. infected	Mean No. worms	No. infected	Mean No. worms
Progamotaenia sp. A	3	2	0	0	0	0	0	0
Progamotaenia sp. B	1	1	0	0	4	3.5	5	0
Triplotaenia sp.	0	0	2	2	1	1	0	0
Cloacina spp.	8	1105	10	231	8	571.3	10	797
L. eugenii	5	966	9	874.4	1	nodule only	0	0
L. longispicularis	0	0	4	82.5	1	40	3	616.7
R. australis	7	552.9	7	457.1	8	587.5	10	2272
M. pearsoni	5	98	5	124	7	61.4	8	72.5
O. kartana	2	10	2	20	3	463.3	6	36.7
Filarinema sp.	2	5.5	0	0	1	1	3	39.3
G. trifidospicularis	5	6	5	9.8	3	2	3	2.3
A. thyogale	10	720	10	720	9	720	2	2
Dipetalonema sp.	4	1	2	1	6	1	10	2.2

1955), *Rugopharynx australis* (Mönnig 1926) and *Filarinema* sp. The species of *Cloacina* were not considered separately as not all of them have yet been described; they include *C. curta* Johnston & Mawson, 1938, *C. petrogale* Johnston & Mawson, 1938, *C. clarkae* Mawson, 1972, *C. smalesae* Mawson 1975, and *C. kartana* Mawson 1975. The numbers of each species or species group from each locality are shown in Table 1.

Filarinema sp. and *Labiostrongylus longispicularis* both appear to be accidental infections as they occur much more commonly in the Grey Kangaroo (*Macropus fuliginosus*) (Desmarest) which grazes on the same pastures as the Kangaroo Island Wallaby. Other species which have been found in the Grey Kangaroo as well as in the wallaby are *Macropostrongylus pearsoni* (rarely), *Globocephaloides trifidospicularis* (not common) and *Rugopharynx australis* (very common in both host species). *Oesophagonastes kartana* is typically found in the oesophagus of the host, and is collected from the stomach only when the infestation is heavy. The oesophagus was not examined regularly, and *O. kartana* may have been present in some of the wallabies though not recorded from the stomach.

Examination of the stomach wall showed that nodules caused by third stage *L. eugenii* larvae[1] were present in all hosts with adult *L. eugenii* in the stomach. It will be noted that the most striking variations in distribution were in the cases of *L. eugenii*, *Dipetalonema* sp., and *A. thylogale*. The incidence of infection with *Cloacina* spp. was high from all four localities, but possibly the actual species within the species group varied.

The territorial behaviour and movement patterns of these wallabies have not been studied although occasional individuals have been recorded moving up to 16 km (Andrewartha & Barker 1969). However, those collected in the present study were probably from different populations, so the observed differences in helminth distribution between the sites represent real differences in the infestations on Kangaroo Island.

Each of the sampling sites had different vegetation and soil types. Rykovskii (1972) suggested a close relationship between these factors and the viability of larval stages of trichostrongyles. Together with the differences in rainfall they may have been important in determining the distribution of the helminths.

The low incidence of *L. eugenii* in hosts from Pioneer Bend and Brookland Park (noted also in other collections from the same area in 1975) is noteworthy because in 1972 and 1973 this species was plentiful there. As mentioned above, the only difference noted in the collection areas between these periods was the use of sprays on the pasture.

Seasonal variation

A survey was made of the total numbers and incidence of the different species of strongyles in the stomach of the wallaby throughout the year. It was hoped to find how the wet winter and hot dry summer affected the different species.

Male wallabies were taken at two monthly intervals from April 1972 to January 1974 from Pioneer Bend and Brookland Park, and subsequently from these and other areas. Until September 1972 our sample comprised four

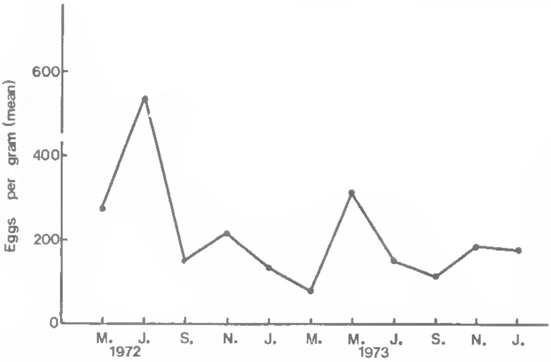

Fig. 2. Mean faecal egg counts: May 1972 to January 1974.

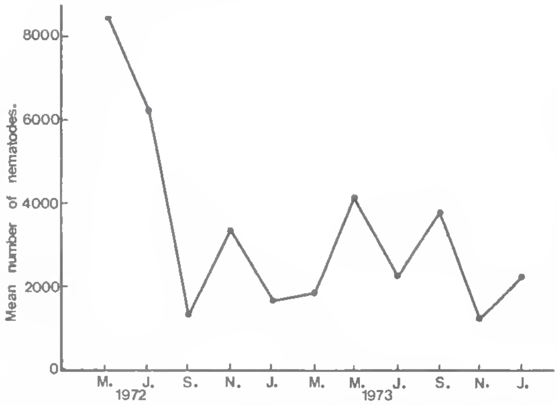

Fig. 3. Total worm burdens: May 1972-January 1974. The figure at each two monthly interval is mean obtained from 4-6 male wallabies.

TABLE 2

Occurrence (% of hosts infected) and relative abundance of nematode species collected from the stomach lumens of 99 wallabies between 1972 and 1975. Abundance expressed as % of largest total number—107527 *Cloacina* spp.

Species	Wallabies infected %	Relative abundance %
Cloacina spp.	98	100
L. eugenii	45	76.2
L. longispicularis	20	1.6
R. australis	84	31
M. pearsoni	34	6.5
O. kartana	43	2.5
Filarinema sp.	4	0.03

animals; subsequently 6 animals were taken. To compare seasonal differences, only the 64 animals from Pioneer Bend and Brookland Park were used. To compare relative abundance of the different spp., 99 hosts from all localities were used.

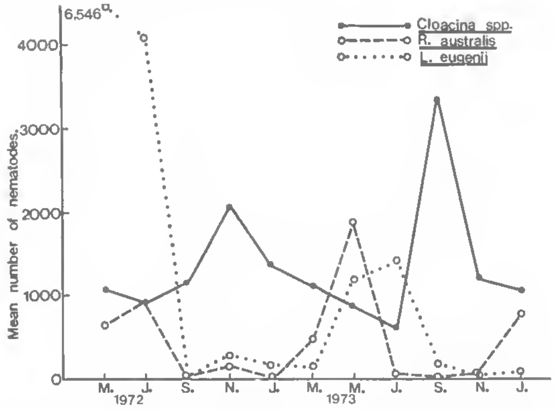

Fig. 4. Mean bi-monthly occurrence of three groups of nematodes, May 1972-January 1974.

At each autopsy a faecal sample was taken and the eggs per gram counted, using a modified Whitlock counting chamber. Faecal egg counts (Fig. 2) show peaks of egg production in May and November of 1972 and 1973, and in March and November 1974. These peaks indicate times when the potential number of infective larvae on the pasture was high.

The stomach of each wallaby was removed and the contents sieved through bolting silk ("64 mesh/inch"), diluting the retained solid material to an appropriate volume (200 or 400 ml), and sampling using Clark et al.'s (1971) method of enabling calculation of worm totals to a S.D. of ±5 worms. All the nematodes in each sample were fixed in hot alcohol, cleared in lactophenol, identified and counted.

Mean total worm burdens were determined for each bi-monthly sample of wallabies (Fig. 3). Highest figures occurred in May and November 1972 and May and September 1973, and lowest in September 1972, January and November 1973. The unseasonably dry weather (see Fig. 1) experienced earlier may have been responsible for the low worm burdens recorded in September 1972. The variation in worm burdens of individual wallabies sampled at the same time was often large. The oldest and youngest wallabies usually had the smallest number of nematodes. Only two young animals which had just left the pouch had no stomach nematodes. The relative abundance of each species was determined by expressing the total number (collected from 99 tammars), as a percentage of the largest total (Table 2). This is to distinguish between a nematode species present in large numbers in a few hosts, and a species present in small to moderate numbers

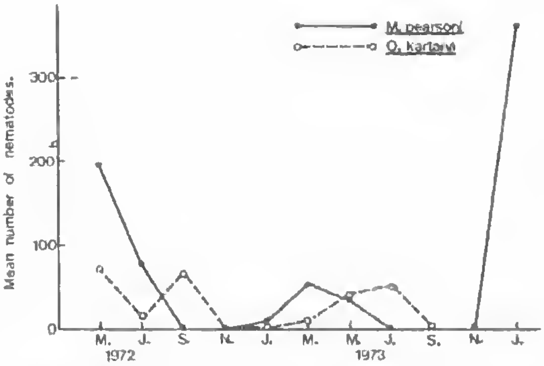

Fig. 5. Mean bi-monthly occurrences of two nematode speices, May 1972-January 1974.

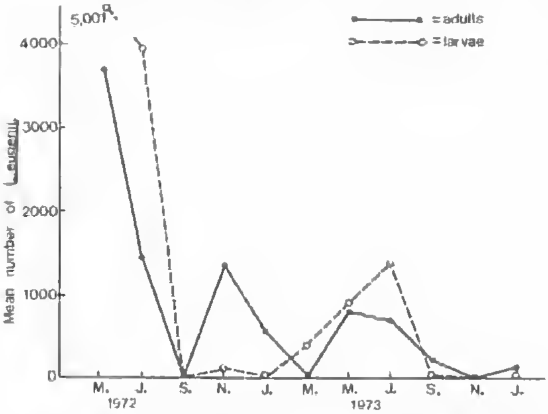

Fig. 6. Mean bi-monthly occurrence of adult and larval *Labiostrongylus eugenii*. Only infected animals were used to obtain the mean.

TABLE 3

Seasonal presence or absence of *L. eugenii* in the stomach lumens of 64 wallabies

	Present	Absent	Total
May 1972 and 1973	7	3	10
July 1972 and 73	7	3	10
Sept. 1972 and 73	3	7	10
Nov. 1972 and 73	2	8	10
Jan. 1973 and 74	3	9	12
March 1973 and 74	8	4	12
Total	30	34	64

χ^2 = 12.53 where χ^2 (5%) = 11.07 and χ^2 (2.5%) = 12.83

in a large number of hosts. Only the more common species, *Rugopharynx australis*, *Macropostrongylus pearsoni*, *Oesophagonastes kartana*, *Labiostrongylus eugenii* and the group of *Cloacina* spp., were considered in greater detail.

The results of the analysis of seasonal difference in abundance of each of these species are shown in Figs 5 & 6. The 1972 figures for *Cloacina* spp. seem to have been influenced by the dry conditions in September of that year. The peak for *M. pearsoni* in January 1974 was due to one host with an extremely heavy infestation. In all, the periods of heaviest infestation occurred during the wet season (*Cloacina* spp. early spring, *R. australis* late autumn and winter, *M. pearsoni* early autumn, *O. kartana* autumn and winter months only, *L. eugenii* late autumn and winter).

Statistical analysis of the data for *L. eugenii* (Tables 3 and 4) gave χ^2 values statistically significant at the 5% level, thus indicating that *L. eugenii* has a cycle of incidence dependent on the seasons. For this analysis counts from March 1974 were included.

The populations of *L. eugenii* from each host were sorted into adults and larval stages (Fig. 6). No third stage larvae and minimum of fourth stage larvae were found in the stomach lumen between September and January. Observations on the life cycle of *L. eugenii* have shown that third stage larvae are most likely to be acquired between June and November of any year. These larvae enter the stomach wall and remain there for about eight weeks or longer if development is inhibited[1]. It is likely that 3rd stage larvae are being ingested during this period and invade the stomach wall. Increasing numbers of these larvae would be migrating into the stomach lumen as 4th stage larvae from February onwards. Comparisons between individual hosts within the same sample revealed heterogeneous age structures, therefore the number of hosts sampled was too small to allow statistical analysis.

TABLE 4

Seasonal abundance of infection with *L. eugenii* in 64 wallabies

	Number of *L. eugenii*				
	a	b	c	d	Total
May 1972 and 73	4	1	2	3	10
July 1972 and 73	4	1	0	5	10
Sept. 1972 and 73	9	1	0	0	10
Nov. 1972 and 73	9	0	1	0	10
Jan. 1973 and 74	10	0	2	0	12
March 1973 and 74	6	3	1	2	12
Total no. wallabies	42	6	6	10	64

χ^2 = 26.77 where χ^2 (5%) = 25

a—0-500
b—500-1000
c—1000-2000
d—2000

Incidence of infestation of *L. eugenii* with respect to host age was also examined. Those wallabies collected before March 1974 were grouped according to age, with 36 1–3 years old in one group and 22 over three years old in the other. Wallabies less than one year old were not considered, as they were never found to harbour *L. eugenii* (though other species might be present). An analysis of the incidence of infection was scored in terms of presence or absence of the species partly because of the small host sample size, and partly because of the practical difficulties involved in estimating the number of 3rd stage larvae in the stomach wall. The results of this analysis indicated that incidence of infection was independent of host age. The host sample was too small to analyse in terms of the age structure of the *L. eugenii* populations. Therefore it was not possible to say whether older wallabies have smaller numbers of *L. eugenii* larvae.

It was found that the times of peak egg production as determined by faecal egg counts appeared to be synchronized with those seasons most advantageous to the nematodes. The peaks occurring in May were at the time when weather conditions for survival of infective larvae were good and, in fact, numbers of infective larvae present on the herbage at this time have been found to be high.[1] The November peak occurred when conditions might have been too dry for many larvae to survive. However, such larvae as did reach infectivity did so at a time when there would be a large number of susceptible hosts (joeys just out of the pouch) grazing the pastures. A build up of infective larvae, from those overwintering as well as from recent hatching, would be possible from late winter through to spring.

The total worm burdens fluctuated both seasonally and from year to year. However, there was a trend towards lowest worm burdens during the dryest periods of the year. A number of workers (Anderson 1972; Gordon 1958; Parnell 1963) have found that sheep living in areas with winter rainfall record highest worm burdens in the late winter and early spring. Thus it appears that the uptake of infective larvae of trichostrongyles by domestic stock as well as strongyles infesting macropods occurs during or just after wet periods. The success of these Australian nematode species is probably related to this synchronisation of their life cycles with the season. The present study showed that the majority of 3rd stage *L. eugenii* larvae are ingested during the winter (older wallabies) and spring (wallabies just leaving the pouch). These develop in the stomach wall and escape into the lumen from February on as fourth stage larvae. They develop to maturity in the stomach lumen, producing eggs from late autumn through the winter. This long prepatent period of about 7–9 months[1] is probably important in enabling the synchronisation of life cycle with changes in seasons.

Seasonal differences in species composition of worm burdens were also observed. However, a detailed analysis of the population structures of species other than *L. eugenii* was not undertaken.

Acknowledgments

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THE AUSTRALIAN FROG CHIROLEPTES DAHLII BOULENGER: ITS SYSTEMATIC POSITION, MORPHOLOGY, CHROMOSOMES AND DISTRIBUTION

BY M. J. TYLER, MARGARET DAVIES & MAX KING

Summary

The external morphology, osteology and karyotype of Chiroleptes dahlii demonstrate that this species is erroneously referred to the Leptodactylidae and is in reality a hylid related to the southeastern Australian species, Litoria raniformis.